ISSN 0973-1555(Print) ISSN 2348-7372(Online) HALTERES, Volume 6, 33-50, 2015

Coprophilic dipteran community associated with horse dung in Malaysia

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Abstract

Blood-sucking flies are known as vectors of pathogens to farm animals worldwide. However, little is known about dipteran biodiversity associated with horse dung in Malaysia which could serve as vectors. Thus, a field trip to a horse farm located in Tanjung Rambutan, Perak, peninsular Malaysia was conducted in 2010. We examined adults and larvae of dipterans associated with 100 horse dung pats. A total of 1480 dipteran specimens from nine families were collected including Sphaeroceridae, Muscidae, Sarcophagidae, Calliphoridae, Sepsidae, Tabanidae, Ulidiidae, Dolichopodidae and Milichiidae. The lesser dung fly (Sphaeroceridae) which consisted of four species was the most abundant dipteran followed by sarcophagids and muscids. Seven species of muscids were collected from the horse farm and three of them were haematophagic namely Musca conducens Walker, 1859, Musca ventrosa Wiedemann, 1830 and Stomoxys calcitrans (Linnaeus, 1758). Fly larvae collected from the dung were raised to adult stage and subsequently identified as M. conducens and Neomyia gavisa Walker, 1859. We reported new locality records for three species of Muscidae in Malaysia namely Pyrellia proferens Walker, 1859, Lispe kowarzi Becker, 1903 (new to peninsular) and N. gavisa Walker, 1859.

Keywords: Coprophilic Diptera, horse dung, Malaysia, new record, Pyrellia proferens, Lispe kowarzi, Neomyia gavisa.

Received: 24 February 2015; Revised: 22 April 2015; Online: 4 May 2015; Published: 5 November 2015.

Introduction

The Diptera, or known as two-winged flies, is one of the largest orders of Insecta and can be found almost ubiquitously on Earth (Triplehorn and Johnson, 2005). Members of Diptera play variety of roles in ecosystem either as pollinators, nutrient recyclers, or carrion decomposers (Borkent and Harder, 2007; Hirabayashi and Wotton, 1998; Putman, 1978). However, there are many dipteran species of economic importance which are responsible for loss of agricultural products and commodity (White and Elson-Harris, 1992; Spencer, 1973). Most importantly, flies have been incriminated either as biological or mechanical vectors to wide range of infectious diseases that are prevalent in both developed and developing countries (Gubler, 2002).

Other than ticks (Acari: Ixodida), Diptera is considered a significant group of arthropods in term of medical and veterinary importance (Cleton et al., 2012). Flies are found accountable for the transmission of variety of pathogens namely viruses, bacteria, protozoans and helminthes (Gestmann et al., 2012), which are responsible for the spread of infectious diseases such as malaria, lymphatic filariasis, dengue, yellow fever, West Nile fever, and chikungunya (transmitted by nematocerans such as mosquitoes), leishmaniasis, onchocerciasis, loiasis (transmitted by sand flies, black flies and deer flies, respectively) (Service, 2012). Other members of Diptera such as house flies (Musca domestica L.), face flies (Musca autumnalis De Geer), dog dung flies (Musca sorbens Wiedemann), and blow flies (Lucilia spp.) are a source of nuisance to humans and animals (Graczyk et al., 2001). Some species may act as the causative agent to myiasis either obligatory or facultative on animal hosts (James, 1947; Hall and Wall, 1995). Besides, there are many dung-frequenting and carrion-breeding dipterans which may spread pathogenic organisms to the surrounding environment, including human dwelling (Howard, 2001).

Other than the diseases mentioned above, veterinary important Diptera have been reported to impose a serious economic loss to the agricultural sector (Bowman, 2014). For instance, In the United States, flies had impacted the cattle production substantially by losing $2,211 million per year (Taylor et al., 2012a). The stable fly, with just 200,000 flies emerging from an average sized winter hay feeding site could reduce annual milk production of 50 dairy cows by an estimated 890 kg (Taylor et al., 2012b).

In Malaysia, Reid (1953) published the first note on the local house flies and blow flies and indicated their important roles as potential mechanical transmitter of pathogens causing human diseases. Subsequently, Sulaiman et al. (1988) demonstrated that the cyclorrhaphan flies collected from four sites in Malaysia are carriers of human parasitic helminths such as Ascaris lumbricoides, Trichuris trichiura, and filariform larva of hookworm (Necator americanus). Sulaiman et al. (2000) trapped four species of synantrophic flies in downtown Kuala Lumpur viz. Chrysomya megacephala, Chrysomya rufifacies (= Achoetandrus rufifacies), M. domestica and M. sorbens. A total of 18 bacterial species was isolated from these specimens, with Burkholderia pseudomallei, the causative pathogen for melioidosis, had been reported for the first time. Nazni et al. (2005) examined bacteria from the house flies and found 10 different species of bacteria (e.g., Bacillus sp., Staphylococcus sp., Proteus sp., Escherichia sp., Klebsiella sp. etc.) isolated from feces, vomitus, external surfaces and internal organs of house flies. However, newly emerged house fly did not harbor any bacteria. The importance of house fly wings in mechanical transmission of Vibrio cholerae was assessed and the results revealed that the wings did not play an important role in pathogen transmission (Yap et al., 2008). Nazni et al. (2013) carried out a study on house fly to establish whether the house flies can transmit the H1N1 virus mechanically. The findings indicated that the persistency of H1N1 virus on fly legs could be detected up to 24 hours either in chilled or actively flying flies. However, virus was not found in the vomitus, feces and the external body of the flies.

Recently, filth fly surveys were conducted in Malaysia by Nurita and Hassan (2013), Khoso et al. (2015) and Heo et al. (2010) where they found a very high diversity of filth flies in the region. Nurita and Hassan (2013) recorded eight dipteran species breeding in the solid waste namely: M domestica, M. sorbens, Synthesiomyia nudiseta Van der Wulp, Hydrotaea chalcogaster (Wiedemann), C. megacephala, Lucilia cuprina (Wiedemann), Hemipyrellia ligurriens (Wiedemann) and Sarcophaga sp.; whereas Heo et al. (2010) recorded 16 families of Diptera from the cow dung. These results showed that landfills and animal farms can act as major breeding grounds for filth flies.

From the above stated data, it is clear that little is known about the coprophilic dipteran community associated with horse manure in Malaysia, particularly the blood sucking flies that might feed on horses. Hence, there is a need to identify the veterinary important Diptera in the horse farm, as the baseline data would be of paramount important in terms of risk management and in vector control programs in future. The present study was aimed to determine the diversity and abundance of coprophilic Diptera associated with horse manure, and to identify the veterinary and medically important dipterans in a horse farm in Malaysia.

Materials and Methods

Horse farm located in Tanjung Rambutan, Perak, Malaysia was selected as the study site (4°41’13” N 101°09’31” E, ~89 m a.s.l.) located approximately 212 km northwest from the capital city, Kuala Lumpur (Fig. 1). A total of four visits to the horse farm were conducted from 8-11 November 2010. Duration and time of fly collection was 5 hours per visit from 9 am to 2 pm. The mean temperature and humidity during the survey period was 28.92 ± 2.36 °C and 73.88 ± 10.72%, respectively. The weather conditions during the four-day field trials were mostly cloudy and all weather data was obtained from the nearest weather station in Ipoh, Perak.

We examined a total of 100 horse dung pats scattered in an area of approximately 5,900 m^2. The age of horse dung examined was from fresh to 3-day old. Each dung pat was examined for the presence of adult flies and dung-breeding larvae. Adult flies either resting on or swarming around the dung pats were collected using a sweep net or transparent plastic bags, which were then transferred to a killing jar which contained cotton balls soaked with ethyl acetate. A pair of forceps was used to examine and recover fly larvae from the dung pats. Any larvae or soil arthropods observed in the dung were then collected and preserved in vials (diameter: 25 mm; height: 85 mm) containing 70% ethanol with label. Several larvae from the same batch were also collected and placed in a transparent plastic container (diameter: 44 mm; height: 57 mm; volume: 70 ml) together with a small amount of horse manure which served as food source for rearing purposes. The screw cap of the rearing container was removed and replaced with a breathable paper towel which had been tighten with a rubber band around the lid. Few drops of water were added ad-libidum to maintain the moisture of the horse manure throughout the rearing periods. All preserved and rearing specimens were then transferred to parasitology laboratory at the Faculty of Medicine, Sungai Buluh Campus, Universiti Teknologi MARA.

The emerging adult flies from rearing containers were prompted for species identification under a stereomicroscope (Olympus SZX7, Japan) by using the keys provided in Triplehorn and Johnson (2005), Emden (1965) and Kurahashi et al. (1997). Several adult specimens from the families Calliphoridae, Muscidae and Sarcophagidae were sent to the third author (Kurahahsi, H.) while specimens of Sphaeroceridae were sent to the fourth author (Hayashi, T.) for species confirmation. Other soil arthropods recovered from horse dung were processed for preservation and identified by using respective taxonomical keys to the lowest taxon. Collembola, ants and centipedes were preserved in vials filled with 70% ethanol while Coleoptera were pinned and labelled. For acari processing, the mite specimens were placed in lactophenol (50% lactic acid, 25% phenol crystal, and 25% distilled water) for clearing purposes. They were left in clearing medium for a week and then transferred on slides by using a probe. Hoyer's medium was used as the mounting fluid and then the specimens were covered with thin cover slips. The slides were then placed in a drying oven at 40°C for a week. The mites were identified to family level based on Krantz and Walter (2009) whereas genus of Macrochelidae was confirmed using the key of Emberson (1980).

Diversity and abundance data of the collected dipterans was calculated for ecological indices (Dominance, Species Richness, Simpson's Index, Shannon-Wiener Index, and Evenness) for the study period. Dominance (D_i) was calculated according to the equation:

$$D_i = \frac{n_i}{N} \times 100$$

Where n_i is the number of individuals collected during the study period, and N is the total number of specimen collected. Species dominance of all families or species were classified according to Tischler's scale: eudominant $10\% \leq D_i \leq 100\%$, dominant $5\% \leq D_i \leq 10\%$, subdominant $2\% \leq D_i \leq 5\%$, recedent $1\% \leq D_i \leq 2\%$, and subrecedent $0\% \leq D_i \leq 1\%$ (Tischler, 1949).

Species richness (S) is the total of different species presented in the sample while Simpson's Index (D) measured both richness and proportion of each species and is calculated by using the formula:

$$D = \sum_{i=1}^{S} P_i^2$$

Where P_i is the proportion of species i. In brief, Simpson's index is the sum of proportion of each species in the community and represented the probability of two randomly selected individuals in the community belong to the same species. Shannon-Wiener Index (H') is similar with Simpson's Index where the measurement takes species richness and proportion of species into account, and is calculated by the following formula:

$$H' = -\sum_{i=0}^{n} P_i(\ln P_i)$$

In general, Shannon-Wiener Index is the negative sum of multiply products between species proportion (P_i) and natural log of species proportion (ln P_i). Evenness (E) is an indicator of similarity in abundance of different species. Evenness is measured on the scale from 0 to 1 where zero represents more variation in communities whereas one represents complete evenness. Evenness is defined as:

$$E = \frac{H'}{\ln S}$$

Evenness is the number obtained via dividing the value of Shannon-Wiener Index by natural log of species richness (S).

Fig. 1. Location of the study site at peninsular Malaysia is indicated by a pink arrow (map created using www.mapbox.com)

Results

A total of 1,480 adult specimens from nine families of Diptera were collected from the horse farm. The families consisted of Sphaeoceridae (four species), Muscidae (seven species), Sarcophagidae (two species), Calliphoridae (two species), Sepsidae (two species), Tabanidae (one species), Ulidiidae (one species), Dolichopodidae (one species) and Milichiidae (one species) (Table 1). Throughout the study period, the most abundant dipterans caught were Sphaeroceridae (93.78%), followed by Sarcophagidae (3.04%), Muscidae (2.30%), Calliphoridae (0.50%), Sepsidae and Ulidiidae (0.10% each) and the least were Tabanidae, Dolichopodidae and Milichiidae (0.06% each) (Fig. 2).

We collected 34 individual of muscids which consisted of seven species (Fig. 3). The species of muscid flies according to their decreasing abundance were as follows: M. domestica (32.4%), Musca conducens Walker (26.50%), Lispe kowarzi Becker (23.50%), Pyrellia proferens Walker (5.90%), Neomyia gavisa Walker (5.90%), Musca ventrosa Wiedemann (2.90%) and Stomoxys calcitrans (2.90%). Out of seven species recovered, three species were blood sucking muscids (M. conducens, M. ventrosa and S. calcitrans), with M. conducens being the most abundant haematophagic species in the horse farm.

Three species of Muscidae were also recorded for the first time in Malaysia namely L. kowarzi (new in peninsular Malaysia), P.

proferens, and N. gavisa (Fig. 4). These adult flies were collected on or around the horse dung pats, indicating their coprophilic behavior in nature. We hereby provide locality notes for all three species:

Specimens examined:

Lispe kowarzi Becker, 1903
MALAYSIA: Perak: Tanjung Rambutan
4°41'13" N 101°09'31"E, ~89 m a.s.l.
9.xi.2010, C.C. Heo, 4 males, 4 females.

Pyrellia proferens (Walker, 1859)
MALAYSIA: Perak: Tanjung Rambutan
4°41'13" N 101°09'31"E, ~89 m a.s.l.
10.xi.2010, C.C. Heo, 1 male, 1 female.

Neomyia gavisa (Walker, 1859)
MALAYSIA: Perak: Tanjung Rambutan
4°41'13" N, 101°09'31"E, ~89 m a.s.l.
10.xi.2010, C.C. Heo, 1 female.

The most abundant Diptera caught in this study was the lesser dung flies (Sphaeroceridae), which constituted about 94% of total specimen collected. This family was observed to frequent on almost every horse dung scattered in the farm, and usually seen in a large group resting on dung surfaces. Four species of sphaerocerids were collected and identified: Norrbomia tropica Duda, Coproica rufifrons Hayashi, Coproica coreana Papp and Coproica aliena Papp (Fig. 5). On the other hand, two species of flesh flies (Sarcophagidae) were found in the farm namely Parasarcophaga taenionota (Wiedemann) and Liopygia ruficornis (Fabricius), and two species of blow flies (Calliphoridae) were identified associated with horse manure, the oriental latrine fly, Chrysomya megacephala Fabricius and the hairy maggot blow fly, Achoetandrus rufifacies (Macquart). These are common blow fly species found in Malaysia and were known to visit decomposing organic matter. Other than that, we also collected specimens from the families Sepsidae, Tabanidae, Ulidiidae (Physiphora sp.), Dolichopodidae and Milichiidae (Milichiella sp.). Note that the tabanid fly is also a blood-sucking species, however, it was rarely seen in the farm.

In addition to Diptera, there were many other taxa associated with horse feces. Examination into the dung pats revealed springtails (one family), beetles (four families), mites (three families), centipedes (one order), ants and spider (one family). However, no earthworm was collected. A butterfly (Lepidoptera: Lycanidae) was also observed to frequent on dung surface. Table 2 listed the families of non-Diptera taxa associated with horse dungs.

Results of ecological indices obtained from formulas stated in methodology were presented in Table 3 and 4. Among dipteran families, family Sphaeroceridae was the most dominant family. Simpson's Index was 0.88 while Shannon-Wiener Index was 0.31. The abundance of all the families collected was dissimilar and skewed, resulted in an uneven distribution among families (E= 0.14). In terms of Family level, only Muscidae was analyzed. Musca domestica, M. conducens and L. kowarzi were all classified in the eudominant group. Simpson's Index and Shannon-Wiener Index were 0.26 and 1.54, respectively, indicated a more equal proportion among species. This observation was strengthened with a higher evenness index (E=0.80).

Discussion

Animal dung pats are patchy but resourceful ephemeral microhabitats which host a vast diversity of species (Hanski and Koskela, 1977). The adults and larvae of Coleoptera and Diptera are considered to play a major role in the dung utilization. However, little is known about the coprophilous fly species compared to studies conducted on dung beetles (Hammer, 1941; Nichols et al., 2007).

Sphaeroceridae are very small, black or brown flies that can be identified by the swollen hind tarsi. They can be found in humid and swampy places near excrement and often aggregated in large number on various mammalian dung piles (Laurence, 1955; Triplehorn and Johnson, 2005). Unexceptionally, the most abundant Diptera collected on horse dung in this study was sphaerocerids. Similar observation was made in U.K. on stable manure and two species of sphaerocerids were considered as potential pests (Hussey, 1957). This finding was in agreement with Bai and Sankaran (1977) where sphaerocerids were found breeding in bovine manure in India. Similar results were demonstrated in North America where sphaerocerids were collected from cattle droppings (Blume, 1985). Other than cattle and horse manures, sphaerocerids can be found in poultry manure (Hulley, 1986). Sphaerocerids, together with acarid mites were also reported as the first colonizer on poultry manure (Stoffolano and Geden, 1987). The cosmopolitan genus Coproica is known to be endemic on various kinds of pasture dung whereas some species breed in decaying vegetable material. In fact, most larvae actually feed on microbial layer developing on the decaying matter (Papp, 2008). However, during the present study, we were not able to collect any sphaerocerid larva in the horse manure. Perhaps it was due to the smaller sample size or other abiotic factors (e.g., condition of horse manure or dung management strategies employed by the farm).

Table 1. Family and species of Diptera recovered from horse dung

Family	Species
Sphaeroceridae	Norrbomia tropica Duda, 1923 Coproica rufifrons Hayashi, 1991 Coproica coreana Papp, 1979 Coproica aliena Papp, 2008
Muscidae	Musca domestica vicina Macquart, 1850 Musca conducens Walker, 1859 Musca ventrosa Wiedemann, 1830 Stomoxys calcitrans (Linnaeus, 1758) Pyrellia proferens Walker, 1859 Lispe kowarzi Becker, 1903 Neomyia gavisa Walker, 1859
Sarcophagidae	Parasarcophaga taenionota (Wiedemann, 1819) Liopygia ruficornis (Fabricius, 1794)
Calliphoridae	Chrysomya megacephala Fabricius, 1794 Achoetandrus rufifacies (Macquart, 1842)
Sepsidae	Unidentified sp. 1 Unidentified sp. 2
Tabanidae	Unidentified sp.
Ulidiidae	Physiphora sp.
Dolichopodidae	Unidentified sp.
Milichiidae	Milichiella sp.

Table 2. Other arthropods associated with horse dungs

Class	Order	Family	Subfamily / Species
Insecta	Collembola	Hypogastruridae	Ceratophysella sp.
	Coleoptera	Scarabaeidae Hydrophilidae Histeridae Staphylinidae	Onthophagus sp. Aphodiinae Unidentified sp. Unidentified sp. Staphylininae
	Hymenoptera	Formicidae	Odontoponera sp.
Arachnida	Mesostigmata	Macrochelidae Uropodidae Parasitidae	Macrocheles sp. Unidentified sp. Unidentified sp.
	Araneae	Lycosidae	Unidentified sp.
Chilopoda	Geophilomorpha	Unidentified	Unidentified sp.

Table 3. Standard ecological indicators of Order Diptera found on horse dung

Family	Number	Dominance	Tischler's scale	P_i	P_i^2	$P_i \ln(P_i)$
Sphaeroceridae	1387	93.7	Eudominant	0.937	0.878	-0.061
Sarcophagidae	45	3.0	Subdominant	0.030	0.001	-0.106
Muscidae	34	2.3	Subdominant	0.023	0.001	-0.087
Calliphoridae	7	0.5	Subrecedent	0.005	0.000	-0.025
Sepsidae	2	0.1	Subrecedent	0.001	0.000	-0.009
Ulidiidae	2	0.1	Subrecedent	0.001	0.000	-0.009
Tabanidae	1	0.1	Subrecedent	0.001	0.000	-0.005
Dolichopodidae	1	0.1	Subrecedent	0.001	0.000	-0.005
Millichidae	1	0.1	Subrecedent	0.001	0.000	-0.005
Total	1480	100.0		1.000		
Ecological Indicators			**Value**			
Species Richness (S)			9			
Simpson Index (D)			0.880			
Shannon-Wiener Index (H’)			0.312			
Evenness (E)			0.142			

Table 4. Standard ecological indicators of Family Muscidae found on horse dung

Species	Number	Dominance	Tischler's scale	P_i	P_i^2	$P_i \ln(P_i)$
M. domestica	12	35.3	Eudominant	0.382	0.146	-0.368
M. conducens	9	26.5	Eudominant	0.265	0.070	-0.352
L. kowarzi	8	23.5	Eudominant	0.235	0.055	-0.340
P. proferens	2	5.9	Dominant	0.059	0.003	-0.167
N. gavisa	1	2.9	Subdominant	0.029	0.001	-0.104
M. ventrosa	1	2.9	Subdominant	0.029	0.001	-0.104
S. calcitrans	1	2.9	Subdominant	0.029	0.001	-0.104
Total	34	100.0		1.000		
Ecological Indicators			**Value**			
Species Richness (S)			7			
Simpson Index (D)			0.256			
Shannon-Wiener Index (H’)			1.538			
Evenness (E)			0.790			

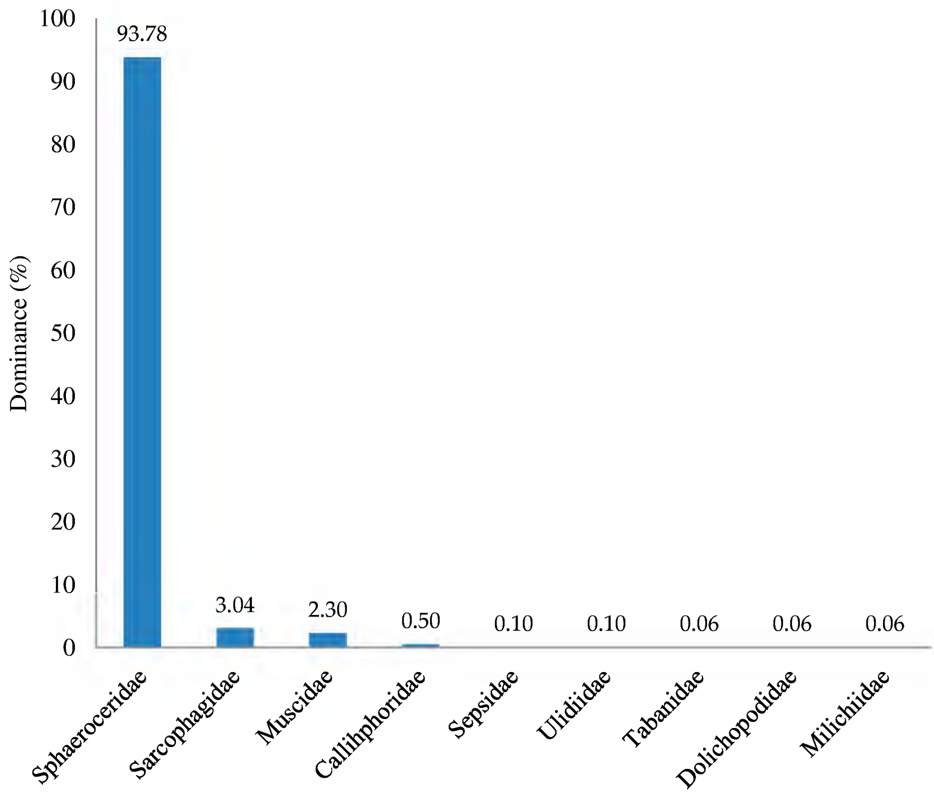

Fig. 2. Abundance of Diptera collected according to family (in percentage)

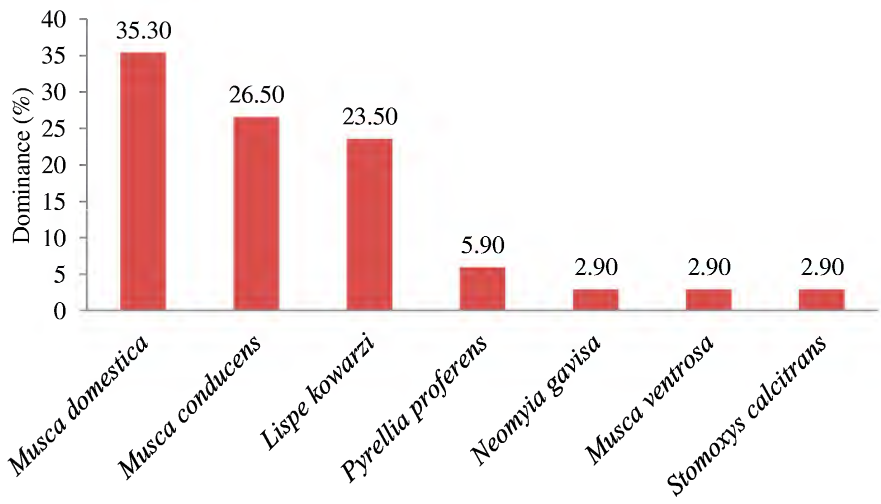

Fig. 3. Species dominance of seven species of family Muscidae collected from cow dung

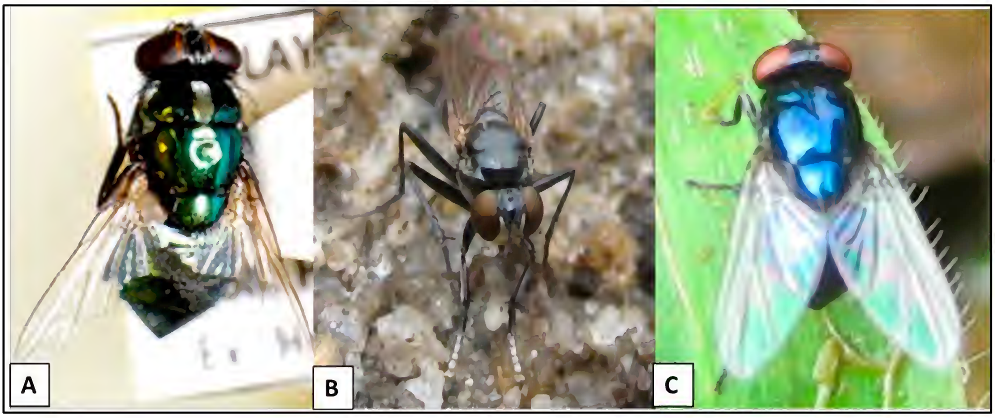

Fig. 4. Newly recorded Muscidae from peninsular Malaysia. A. Pyrellia proferens; B. Lispe kowarzi; C. Neomyia gavisa

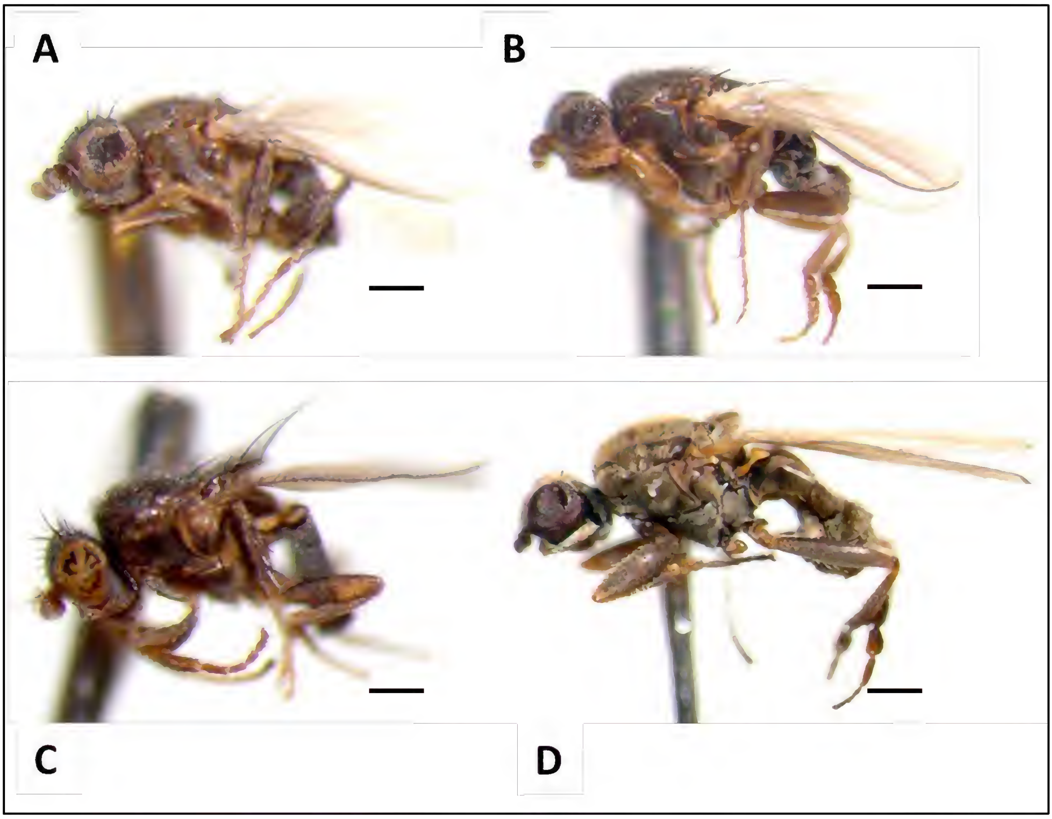

Fig. 5. Sphaeroceridae collected from horse dung. A. Coproica aliena; B. Coproica coreana; C. Coproica rufifrons; D. Norrbomia tropica. Scale bar = 0.5 mm for all figures

We collected four species of blood-sucking flies (three in Muscidae and one in Tabanidae) during the study period. Among these, M. conducens was the most abundant (26.5% of total muscid caught), followed by M. ventrosa and S. calcitrans (both were equally abundant), and lastly a Tabanus sp. As discussed earlier, the blood sucking Diptera are considered medical and veterinary important pests due to their potential in causing and transmitting vector-borne pathogens. Musca conducens was incriminated as the vector for stephanofilariasis, which had been reported to cause chronic eosinophilic dermatitis in scrotal area among Charolais bulls in France (Watrelot-Virieux and Pin, 2006) and transmit Stephanofilaria assamensis in India and Russia (Patnaik and Roy, 1969; Johnson et al., 1981) and Stephanofilaria kaeli in peninsular Malaysia (Fadzil, 1973). Interestingly, we collected 13 larvae of M. conducens from horse manures and reared them to the adult stage, out of these four males and nine females emerged (male: female ratio 1:2).

Musca ventrosa was found in fresh markets in Malaysia, albeit its population was low (Khoso et al., 2015). Nazni et al. (2007) conducted a study on distribution of diurnal and nocturnal dipterous flies in Putrajaya, Malaysia and collected six specimens of M. ventrosa out of 1,534 flies (0.04%) during the day. Despite feeding on wounds, sores and bites inflicted by other insects, these flies also frequent cow dung and their larvae breed in it (Patton, 1922). So far, little literature is available on the status of M. ventrosa as a vector for veterinary diseases although it is a haematophagous species (Sucharit and Tumrasvin, 1981). In our study, we collected a single adult M. ventrosa. Due to their low population, their feeding impact on horses seems to be limited.

Adults of S. calcitrans suck blood and inflict painful bites and thereby causing trouble to the confined livestock such as cattle and horses (Service, 2012). The fly causes interference with the normal feeding activity of cattle which could result in weight loss, decreased milk production, and possibly anemia. Moreover, stable flies have been found responsible for the mechanical transmission of several livestock diseases such as equine infectious anemia and bovine anaplasmosis (Bay and Harris, 1988). Likewise, we collected only one adult S. calcitrans, which may suggest limited vectorial capacity due to their low population density.

Heo et al. (2010) conducted a study on cow dung Diptera in Kuala Lumpur, Malaysia and found Musca inferior Stein to be the most abundant (37%) blood-sucking muscid associated with cattle manure, followed by S. calcitrans (10.3%), M. ventrosa (9.4%), M. crassirostris (3.4%) and Haematobia sp. (0.8%). In comparison with the present study, the results showed that M. ventrosa and S. stomoxys were present in both study sites and were of intermediate abundance either on cattle or horse manures. However, we did not collect M. inferior, M. crassirostris and Haematobia from the horse dung. Additionally, the most abundant non-biting Diptera associated with cattle dung was Sepsidae, which was different in the current study. A comparison of dipteran composition with Heo et al. (2010) is presented in Table 5.

Although cattle and horses are herbivores, there are some remarkable differences in the diversity of arthropod assemblages on their wastes. The differences in results of arthropod communities between cattle and horse dungs could be due to two main reasons: biotic and abiotic factors. Biotic factors include the differences in microbiome in the animal dung (Dowd et al., 2008; Girija et al., 2013; Costa and Weese, 2012), which may affect directly the behavior of arthropods (Ezenwa et al., 2012) or perhaps the subsequent colonization events (Shade et al., 2013). The microbiome on the dung could be the direct result from the animal intestine itself or the contamination from the external environment (Girija et al., 2013). Besides, arthropod visiting the dung could also deliver and contribute microbes to it (Estes et al., 2013) which consequently initiate a change in arthropod successional sequence either due to the exploration of new resources (Pechal et al., 2014; Finley et al., 2015) or interkingdom signaling through volatile organic compounds released by bacteria (Tomberlin et al., 2012). Moreover, ecological interactions such as mutualism, competition, predation and parasitism could shape the structures and functions of the microbial and arthropod communities in the dung ecosystem (Valiela, 1974). The abiotic factors include the differences in spatial and temporal factors (e.g., geographical locations of the study sites and timing of study), weather factors (e.g., differences in temperature and relative humidity, event of rains), and most notably, is the difference in the physical structure and chemical composition between cattle and horse manures. Cattle dungs had more moisture content (31%) than horse (27%) (Akhtar et al., 2013). Besides, horse dungs were more alkaline (pH 9.4) compared to cattle (pH 5.2). The percentage of organic carbon, C:N ratio, total nitrogen and concentration of inorganic nitrogen were generally higher in cow dung in comparison with horse manure (Ajwa and Tabatabai, 1994).

Table 5. Comparison of dipteran composition of cow and horse dungs.

Result	**Cow dung (modified from Heo et al., 2010 with correction on the species of the Diptera larva collected)**	**Horse dung**
Location of study	Sentul Timur, Kuala Lumpur (3°11’ N 101°41’ E)	Tanjung Rambutan, Perak (4°41’13” N 101°09’31” E)
Total family collected	16	9
Most abundant Diptera	Sepsidae (43.8%)	Sphaeroceridae (93.8%)
Total species collected in Muscidae	12	7
Total blood sucking species	6	4
Percentage of haematophagous muscid collected	51.5%	29.4%
Most dominant haematophagous muscid	M. inferior	M. conducens
Diptera larvae collected from dung	M. inferior Stein Psychodidae	M. conducens N. gavisa

Neomyia spp. is common muscids to frequent on animal dung. Their larvae are found to be saprophagous, coprophagous (Couri et al., 2006) and also serves as food source to many other carnivorous larvae living in the dung (Emden, 1965). The larvae of Neomyia and other dung fauna (e.g., dung beetles and earthworms) play a vital role in dung decomposition and nutrient recycling (Holter, 1977, 1979; Sommer et al., 2001). In Malaysia, six species of Neomyia have been documented to date namely Neomyia rufitarsi (Stein), Neomyia coerulea Wiedemann, Neomyia coeruleifrons (Macquart), Neomyia diffidens (Walker), Neomyia lauta Wiedemann and Neomyia indica Robineau-Desvoidy (Emden, 1965; Heo et al., 2010). With the finding of N. gavisa during the present study, the number of species of Neomyia in Malaysia has increased to seven. Neomyia gavisa was recorded in Sichuan Province, China as dominant species and can be seen all year round (Wang and Feng, 2008). To date, no literature is available about the role of Neomyia in disease transmission.

Pyrellia proferens, a new record from Malaysia, was previously documented from India, Myanmar and Indonesia. The adults can be found on flowers and on decaying animal matters, especially on dungs. The larvae live in animal excrement and feed on carrion (Emden, 1965).

Lispe spp. are shore-living mucids and their larvae are aquatic in the environment (Shinonaga and Kano, 1989; Pont et al., 2012). The adults Lispe are known to be highly predacious to small insects and potentially useful in biological control of black fly (Werner and Pont, 2006). We had a chance to collect L. kowarzi through this study and it is therefore newly recorded in peninsular Malaysia. It was previously recorded in Kota

Kinabalu, Sabah, Malaysian Borneo (Vikhrev, 2012a). In general, this species is widely spread in South Palaearctic and Oriental region (Tumrasvin and Shinonaga, 1982; Ebejer and Gatt, 1999; Bharti, 2008; Vikhrev, 2012a). It is interesting to note that this species could be the predator to other small insects associated with horse manure. So far, six species of Lispe (Lispe pacifica Shinonaga and Pont, Lispe assimilis Wiedemann, Lispe pectinipes Becker, Lispe manicata Wiedemann, Lispe orientalis Wiedemann) have been recorded in Malaysia, including the newly recorded L. kowarzi (Shinonaga and Pont, 1992; Nazni et al., 2007; Kurahashi and Shinonaga, 2009; Chew et al., 2012; Vikhrev, 2012b).

House flies, M. domestica, are responsible for the spread of infectious diseases such as typhoid, dysentery, diphtheria, leprosy, tuberculosis, intestinal parasitic infections in humans and are found to be mechanical vectors for various pathogenic bacteria, viruses and protozoans (Greenberg, 1973; Chaiwong et al., 2014). Blow flies such as C. megacephala and A. rufifacies may act as facultative myiasis agents on the wounds of animals inflicted by injuries or insect bites (Sukontason et al., 2005). Moreover, C. megacephala has been shown to have greater chances of finding helminth ova attached to their external surfaces compared to M. domestica. A study conducted in Ethiopia demonstrated that A. rufifacies acted as a vector of at least five helminthes parasites and four species of protozoan parasites (Getachew et al., 2007). It is pertinent to mention here that these two species of blow flies are forensically important in Malaysia and other adjacent countries and their larvae are frequently encountered in forensic cases (Sukontason et al., 2002; Lee et al., 2004; Chen et al., 2004; Wang et al., 2008).

Parasarcophaga taenionota and L. ruficornis have been found to breed on human and animal carcasses in Thailand and Malaysia (Sukontason et al., 2007; Tan et al., 2010; Kumara et al., 2012) and are used by forensic entomologists in determining the minimum post-mortem interval (mPMI) of a corpse. In medicine, the larvae of L. ruficornis have been documented to cause myiasis in the vagina of a comatose woman in Thailand (Sucharit et al., 1981). Both P. taenionota and L. ruficornis had been recently collected at several fresh markets in Malaysia, indicating their synantrophic behaviors that could contribute as potential mechanical vectors of pathogens (Khoso et al., 2015). Moreover, Heo et al. (2010) collected L. ruficornis from cattle droppings, implying its coprophilous behavior.

Dung beetles (Coleoptera: Scarabaeidae) play a crucial role in dung decomposition and improve nutrient recycling and soil structure (Hanski and Cambefort, 1991). Both adults and larvae are dung feeders, and through the process of feeding, dung beetles facilitate a range of ecosystem services such as secondary seed dispersal, bioturbation, parasite suppression, plant growth enhancement, and soil fertilization (Nichols et al., 2008). Higher diversity of dung beetles could accelerate nitrogen and carbon transfer from the grass-produced dung to the soil (Yoshihara and Sato, 2015). Predators such as rove beetles (Staphylinidae), hister beetles (Histeridae), centipedes, spiders and ants were also found in the horse manures. These predators were searching for fly eggs or insect larvae that were residing in the dung (Triplehorn and Johnson, 2005). Predatory staphylinids inflict heavy mortality on fly eggs, maggots, pupae and adults (Valiela, 1969). In tropical rain forest in Sarawak (Malaysian Borneo), Hanski (1983) revealed 60 Scarabaeinae and four Hybosorinae (necrophagous subfamily of Scarabaeidae), about 20 Hydrophilidae, six Histeridae and more than 150 Staphylinidae from dung and carrion. The water scavenging beetles (Hydrophilidae) are coprophagous, but their larvae are predators (Bøving and Henriksen, 1938).

Apart from Insecta, we also collected mites (Acari: Mesostigmata) from the family Macrochelidae, Parasitidae and Uropodidae, with macrochelids being the most abundant inhabitant of the horse dung. The result of the present study is similar to the earlier study by Axtell (1963) where Macrochelidae, Uropodidae, Parasitidae, Oribatidae and Laelapidae were collected from 211 samples of domestic animal manures (e.g. cattle, horses, sheep, chickens and ducks). Mesostigmata are free-living predators and known to predate on soil nematodes, Collembola and insect larvae (Koehler, 1999). Macrochelidae are active consumers of eggs and larvae of synantrophic Diptera and they might be useful as biological control for dung breeding flies (Krantz, 1983). Macrochelid mite can be found especially on coprophagous Scarabaeidae (e.g., Onthophagus sp. and Aphodius sp.) (Glida and Bertrand, 2002). Both Parasitidae and Uropodidae are predacious on other soil arthropods and are closely associated with dung beetles through phoresy (Mašán and Halliday, 2009).

Numerous individuals of collembolans (Hypogastruridae: Ceratophysella sp.) were collected from the horse dung where they occurred in large aggregation. The members of Hypogastruridae feed on the decaying plant materials (Greenslade and Ireson, 1986). They may also be feeding on nematodes and other microscopic animals associated with decomposition (Chernova et al., 2007). It should be noted that Ceratophysella spp. are beneficial as they contribute to the decomposition of organic matter (e.g., carrion and dung) and enhance nutrient recycling in agricultural land (Zhang et al., 2012).

In conclusion, four species of blood sucking flies were identified in the horse farm in Malaysia with M. conducens being the most abundant haematophagic muscid. The most populous non-biting Diptera in the farm was the sphaerocerids. Proper management of animal manure is highly recommended to reduce population of blood sucking muscids and tabanids. Annual trapping and phenological studies of horse dung Diptera should be carried out to draw a clearer picture of the bionomics of veterinary important flies, as well as those dung decomposers who play a vital ecological role in dung ecosystem. Spatial-temporal distribution of coprophagous dipterans communities and their ecosystem functions should be better understood for more efficient Integrated Pest Management (IPM) programs. We also suggest further studies on vectorial capacity and efficiency of certain dung associated Diptera (e.g. M. ventrosa) to determine their possibility in transmitting medical and veterinary pathogens.

Acknowledgements

We would like to express our deepest gratitude to the management staff and veterinarian of the horse farm who granted permission to us for collecting and studying the dung associated arthropods in their facilities. Thanks to Mr. Haji Sulaiman Abdullah (Faculty of Medicine, UiTM) for his kind assistance in the field work. We would like to thank Dr. Frans Janssens (University of Antwerp, Belgium) for Collembola identification and Mr. Edward G. Riley (Texas A&M University, USA) for his consultation on beetle identification from pictures. All laboratory facilities provided by the Faculty of Medicine, Universiti Teknologi MARA are greatly acknowledged. Authors also express sincere gratitude to Dr. Meenakshi Bharti and other anonymous reviewer for reviewing the manuscript.

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